CLXXV. "DIET 4" FOR BREEDING RATS FOR WORK ON VITAMIN A.

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(Received July 14th, 1930.)

THE technique concerning breeding rats is described in detail in a previous publication [Gudjónsson, 1930]. As demonstrated there it was found that in several of their functions the rats were subject to seasonal variations. These variations appeared to have influence on the fore-period of the experimental rat, and their effect could be traced in the after-period, where they may be of decisive significance. The natural consequence of these findings was to try to improve the technique so that these sources of error were diminished or avoided. It did not seem unlikely that variations in the vitamin A content of the food given to the breeding rat might be one of the reasons for the variations in the experimental rats.

"DIET 4" FOR BREEDING RATS.

It was therefore attempted to construct a diet for the breeding animals which should be adequate for the normal well-being of the animals, should vary as little as possible in its vitamin A content in the different seasons, and should contain the smallest adequate amount of this substance. Regardless of all theories I have composed the following diet for breeding rats:

Skimmed-milk powder	...	...	...	...	30 %
Rice flour	...	...	...	...	40 %
Autolysed yeast	...	...	...	...	15 %
Hardened coconut oil and shark-liver oil	...	...	...	...	15 %

The skimmed-milk powder was made from pasteurised milk. It contained only about 1 % fat, and should therefore contain only a minimum of vitamin A, or be entirely free of this substance. I also reckoned on it being poor in vitamin B. There is no need to take vitamin C into account. The rice flour is made from polished rice. The top yeast was autolysed at 40° and used without drying. The coconut oil contained 0.3 % shark-liver oil.

The intention was to give the rats a limited supply of vitamin A in a food mixture that would be adequate in other respects, so that rats could be bred which at the age of 30 days were not provided with different or much too great reserves of vitamin A. Their fore-period would then be shorter and more uniform than has been the case in previous experiments. By this means, the breeding of the animals was really made a part of the test system; the fore-

period was not only extended back to the birth of the rat but actually made to cover its descent. In addition, the stock of breeding animals that was raised on this diet was derived from only a few individuals.

In this way I have succeeded in creating a breeding stock that produces experimental material of very uniform quality. The diet proved to be perfect in every respect and did not call for anything in addition but water. The rats ate it well. The young rats thrived on it and grew to normal size; there was never any sign of deficiency disease. This diet has been given to all the breeding animals for three years; now the 10th generation is thriving on it.

Some provisional results with this diet have already been given [Gudjónsson, 1930]. Since then a considerable number of experiments have been carried out with young rats whose parents had been fed on "Diet 4." The following is a summary of the results.

FERTILITY OF RATS ON "DIET 4."

On this diet the fertility is somewhat less than it was on the old diet. Formerly there were about 50 litters a month per 100 females; on "Diet 4" about 40. A contributory cause of this fall is undoubtedly to be sought in the fact that at one time—about 1 year ago—several of the stock rats were getting too old.

Fig. 1 gives the curve for the number of litters per 100 females during the year (number of litters 312, mothers per month 67).

Fig. 1.

Fig. 2.

Fig. 1. Number of litters per 100 females on "Diet 4" in a breeding stock of about 40-50 females.
Fig. 2. Number of young per 100 females on "Diet 4" in a breeding stock of about 40-50 females.

Fig. 2 shows the number of young per 100 females during the year.

Fig. 3 shows the curve for litter size throughout the year. Here the variations are much smaller than among the rats on the old diet.

Fig. 3.

Fig. 4.

Fig. 3. Size of litters cast by females on "Diet 4" in a breeding stock of about 40-50 females.
Fig. 4. Average weight of 30 days old rats of "Diet 4" stock, tabulated according to month of birth. Number of rats about 1000.

"DIET 4" AND THE FORE-PERIOD.

(The fore-period means the period during which young rats get vitamin A-free diet until they begin to show typical symptoms of avitaminosis A.)

Investigation was also made into the average weight of the young rats at the age of 30 days, the length of the fore-period and the increase in weight during the fore-period in the different seasons.

Fig. 4 gives the average weight curve throughout the year for 30 days old rats. The observation period is 3 years. Generally the animals now weigh more than they did before on the old diet. This speaks for the quality of the diet.

Fig. 5 shows the average length of the fore-period.

Fig. 5.

Fig. 6.

Fig. 5. Average length of fore-period in weeks among about 900 rats on vitamin A-free diet. All the rats came from mothers on "Diet 4."

Fig. 6. Average weight variation in the whole fore-period (figures along the ordinate), calculated in percentage of the initial weight, among about 900 rats on vitamin A-free diet. All the rats come from mothers on "Diet 4."

Fig. 6 gives the curve for the average weight variation throughout the fore-period. It is much flatter than the corresponding curve in the previous communication relating to breeding rats on ordinary diet. This and the last curve deviate markedly in a favourable direction from corresponding curves in the afore-mentioned paper, section 1. Here the individual variations in the rats are quite minimal; they were wide in the material studied before. The curve seems to show a tendency to greater increase in weight during the fore-period in the months August to January than in the spring and early summer months.

Here the average length of the fore-period for the whole year is about 4.4 weeks as against 8 weeks for animals on the old diet. Here the longest fore-period was 7.5 weeks; in the other animals more than 12.5 weeks. One would probably advance yet further towards an improved and uniform animal material by reducing all the litters to a certain number of young, 6, for instance, as is done by Macy, Outhouse, Long and Graham [1927].

With the breeding stock, diet and breeding technique employed for this material there is attained what was demonstrated in the previous paper to be the most essential object, *viz.* the production of an animal material for vitamin A tests in which all the rats have a favourable and uniform fore-period. This alone makes the results of the tests reliable.

In addition, the shortening of the fore-period is a great advantage as far as time, work and expense are concerned.

On this diet, all the 30 days old rats have probably about the same reserves of vitamin A. It does away with the necessity in Zilva and Miura's method [1921] for bringing the rats to a clinically manifest stage of avitaminosis A. It is even probable that these rats may be used for vitamin A tests as soon as they are 30 days old; and this will perhaps turn out to be the golden mean between the curative method and the preventive method. It may be, as suggested in the previous paper, that it will be possible to estimate the vitamin A content of a substance by the weight curve for the rats before they begin to fall off in normal weight-increase, the length of time being measured during which the reserves of the rat plus the added substance can keep up the normal weight-increase. It might then be possible to use some other standard instead of the weight—for instance, the time it takes for definite symptoms to develop, etc.

SUMMARY.

1. A description of "Diet 4" for rats kept for breeding purposes is given.
2. Seasonal variations, which are present in rats kept for breeding purposes and experimental rats fed on uncontrolled diet, have almost disappeared in rats fed on "Diet 4."
3. The rats breed well on this diet. The young rats are heavier than those bred on other diets and they are more suitable for vitamin A experiments. Experimental animal material of uniform and favourable quality for vitamin A experiments can be produced on "Diet 4", thereby ensuring that the assay of vitamin A by animal experiments becomes a matter of much greater certainty than hitherto.

REFERENCES.

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